Substituent Effects on the Dehydration of Arene Hydrates in Aqueous Solution

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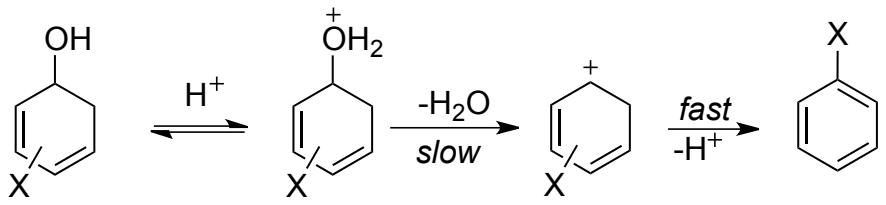
Abstract: Rate constants have been determined by UV spectrophotometry at 25 °C for the acid-catalyzed dehydration of a range of monocyclic benzene hydrates substituted at the 1-, 2- or 3-positions. General acid catalysis was not observed and linear plots of pseudo first order rate constants for dehydration against hydronium concentration were obtained. A Hammett plot of the second order rate constants for acid-catalyzed dehydration, k_H ($M^{-1}s^{-1}$), of unsubstituted **1a** and 1- or 3-substituted benzene hydrates **8a-d**, **14a** and **14c** shows an excellent correlation with σ^+ values and yields a large negative ρ-value of –6.5. The results are consistent with rate determining formation of a benzenium ion in which direct mesomeric interaction with the substituent occurs, presumably permitted by the coplanar arrangement of the diene and carbocation centre in the carbocation intermediate. Data points for 2-substituted hydrates **13a-d** deviate negatively from the Hammett plot as direct mesomeric interaction with the substituent is not possible in the corresponding benzenium intermediates.

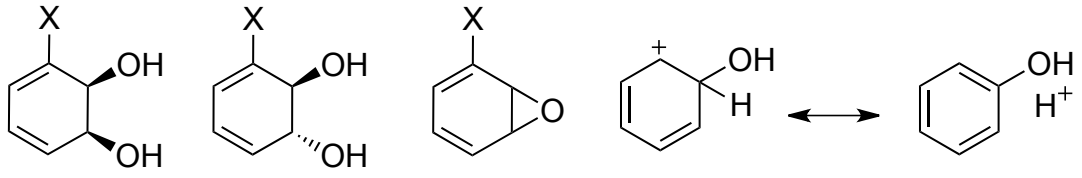
Arene hydrates **1**, formally considered as water adducts of the corresponding arenes **2**, may be accessed by chemical or enzymatic synthesis.[1] The isolations and purifications of the monocyclic hydrates **1** are generally complicated by a rapid dehydration reaction back to the arene. The mechanism for acid-catalyzed dehydration of the parent benzene hydrate **1** (X = H) is well-established and involves the generation of a benzenium intermediate **3** as shown in Scheme 1.[2] The mechanism of this reaction is similar to that for acid-catalyzed dehydration of arylalkyl alcohols.[3] Carbocation formation is rate-determining for the reaction of arene hydrates **1**, whereas the deprotonation of the carbocation intermediate to non-aromatic alkene products is the slower rate-limiting step for the dehydration of arylalkyl alcohols. In the case of the carbocation **3** formed from hydrates **1**, deprotonation to the aromatic product is fast and non-rate limiting.

Scheme 1



1 **3** **2**

The arene hydrates **1** are closely related to other oxidation products of arenes including the *cis*- and *trans*-1,2-dihydrodiols **4** and **5**, respectively, and oxides **6**. These molecules also undergo acid-catalyzed dehydration by analogous mechanisms to that shown in Scheme 1. The second-order rate constants for specific acid-catalysed dehydration of benzene hydrate, oxide and *cis*-dihydrodiol decrease in the order k_H = 190, 32 and 0.11 $M^{-1}s^{-1}$, respectively.[2, 4] In a kinetic study of the dehydration of a large series of 3-substituted benzene-*cis*-1,2-dihydrodiols **4**, Boyd and More O'Ferrall observed a large negative Hammett ρ-value of –8.2 consistent with reaction *via* an electron-deficient carbocation intermediate.[4]



4 **5** **6** **7a** **7b**

Recently, More O'Ferrall and co-workers have provided good evidence for aromatic hyperconjugative stabilization of the β-hydroxybenzenium intermediate **7a** formed in the dehydration of benzene-*cis*-1,2-dihydrodiols **4** (X = H), which they have described as 'hyperaromaticity'.[5] They proposed that the no-bond resonance structure **7b**, associated with hyperconjugation, is subject to particular stabilization as a result of the aromatic character. Evidence for 'hyperaromaticity' included the high *cis*/*trans* activity ratios for reaction of benzene *cis*- and *trans*-1,2-dihydrodiols (k_{cis}/k_{trans} = 4500).[5f] Both dihydrodiols might have been expected to react *via* a common planar carbocation intermediate and, on this basis, very similar reactivities of no more than 2-3 fold based on different reactant stabilities should be observed. The slower reaction of the *trans*-1,2-dihydrodiol compared with the *cis*-analogue was suggested to result from the formation of a benzenium intermediate with a different initial conformation, in which a hydroxyl group rather than a hydrogen occupies the axial position required for optimal 'aromatic' hyperconjugation. This conformation effect is based on the protonated leaving group (OH_2^+) departing in a pseudo-axial position. The k_{cis}/k_{trans} ratios were found to decrease with decreasing aromaticity of the ring formed in the dehydration product (benzene > naphthalene > phenanthrene) *i.e.* upon benzannelation of the double bonds of the reactant. Furthermore, the greatest difference from the normal k_{cis}/k_{trans} reactivity ratio observed for the formation of hyperconjugatively-stablilized non-aromatic carbocations is for hydrates formed from the most aromatic arenes. More O'Ferrall proposed that hyperaromaticity could also explain the lower reactivity of benzene oxide towards dehydration compared with benzene hydrate despite the fact that acid-catalyzed C-O bond breaking in a normal epoxide occurs more ~10^6-fold readily than in a structurally related

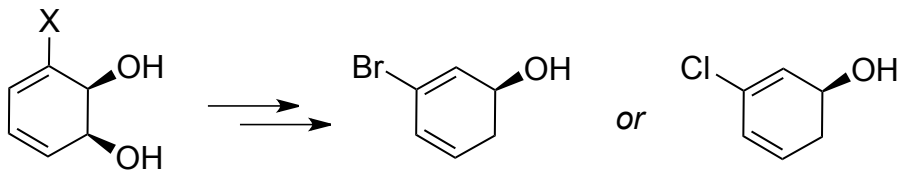
alcohol.[5c] In 2012, More O'Ferrall and co-workers reported the first example of a base-catalyzed mechanism for the dehydration of benzene *cis*-1,2-dihydrodiols in concentrated hydroxide solution.[6]

Although the mechanism of acid-catalyzed dehydration of the parent benzene hydrate **1** (X = H) is well-established,[2] there have been no reported studies of substituent effects on this reaction. Herein, we report the synthesis of a range of monosubstituted arene hydrates **1** and the kinetic analysis of the corresponding dehydration reactions. These results are compared with analogous data for the 3-substituted-*cis*-1,2-dihydrodiols **4**.

RESULTS

Synthesis As recently described by Boyd, Stevenson and co-workers, arene hydrates have long been implicated as metabolites of arenes in animal cells, however these studies largely refer to the more stable hydrates formed from polycyclic arenes and dihydroarenes.[1b] Apart from the arene hydrate of acetophenone **1** (X = $COCH_3$), there have been no reported examples of the isolation of hydrates of monocyclic arenes from bacterial metabolism, although corresponding *cis*-dihydrodiols **4** have been isolated in such biotransformations.[1b, 1c] Boyd and Stevenson, in their recent report, present the first more generally applicable chemoenzymatic route to arene hydrates.[1b] *Cis*-dihydrodiol bacterial metabolites **4** of monocyclic arenes were used for the synthesis of a range of enantiopure 3-substituted 1-(*S*)-arene hydrates. Our samples of hydrates **8b-c** were prepared by this route (Scheme 2) and were generously provided by Boyd.

Scheme 2

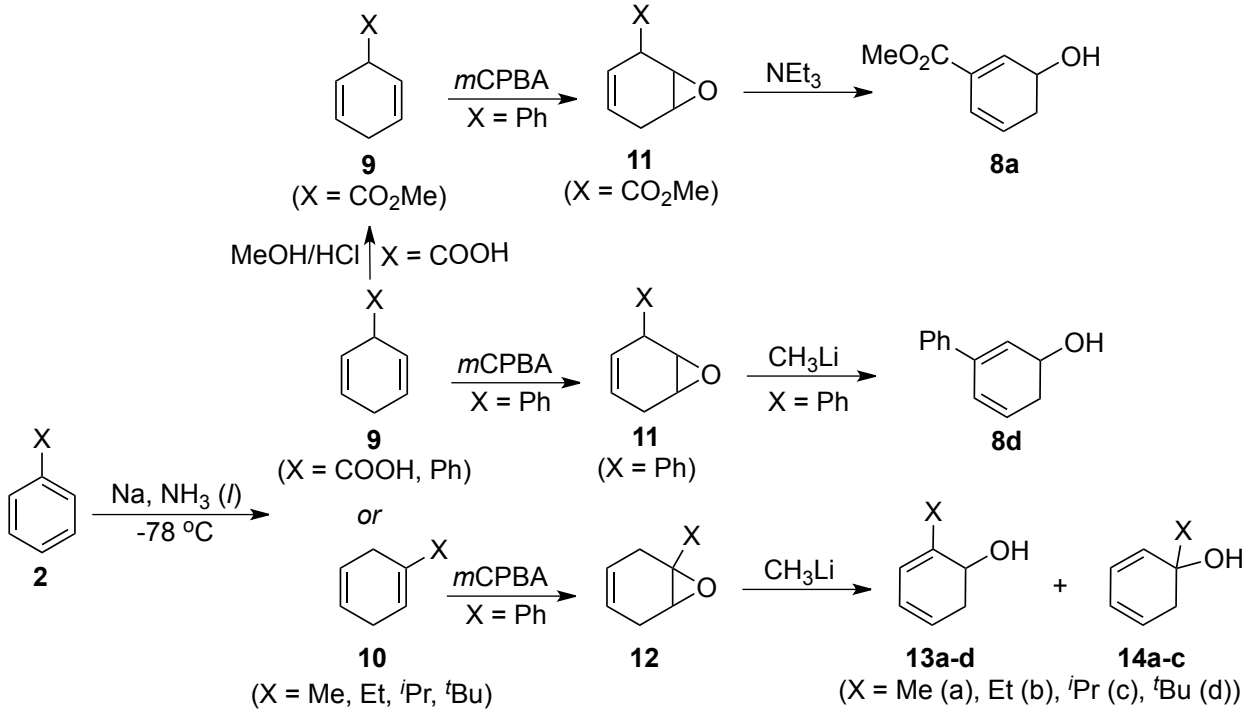


4 **8b** **8c**

The remaining hydrates used in the present study were prepared following a route based on that reported by Starascik and Rickborn[1d] for the synthesis of benzene hydrate **1a** (X = H): Birch reduction of the relevant arene was followed by mono-epoxidation of the resulting 1,4-cyclohexadiene and subsequent ring opening of the epoxide in the presence of a suitable base (Scheme 3). Methylbenzoate hydrate **8a**, had been previously prepared by this route.[7] In this case, Birch reduction of benzoic acid **2** (X = COOH) yielded diene **9** (X = COOH), which was converted in methanolic sulfuric acid to 3-methoxycarbonyl-1,4-cyclohexadiene **9** (X = CO_2Me). Monoepoxidation with *meta*-chloroperbenzoic acid (*m*-

CPBA) yielded epoxide **11** (X = CO_2Me) and subsequent deprotonation[8] using a weak base yielded the hydrate **8a**, which could be easily purified by column chromatography.

Scheme 3



There are no reported syntheses of phenyl or alkyl substituted monocyclic hydrates to our knowledge. Birch reduction of biphenyl **2** (X = Ph) yielded 3-phenyl-1,4-cyclohexadiene **9** (X = Ph), whereas the alkylbenzenes yielded 1-substituted-1,4-cyclohexadienes **10** (X = Me, Et, ^{i}Pr, ^{t}Bu). In general, the dienes were carried through the epoxidation step without prior-purification as more ready purification was achieved after this step. Ring opening of the monoepoxide of 3-phenyl-1,4-cyclohexadiene **11** (X = Ph) by methyl lithium yielded 3-phenyl hydrate **8d**, which was stable enough to purification by flash silica column chromatography. Ring opening of the mono-epoxides **12** (X = Me, Et, ^{i}Pr, ^{t}Bu) yielded an oily isomeric mixture of 2-substituted hydrates **13a-d** and 1-substituted-'*ipso*'-hydrates **14a-d**. All of the alkyl-substituted hydrates **13a-d** and **14a-d** proved unstable to separation and further purification by standard distillation and chromatographic methods. In the case of these

alkyl hydrates, it was decided to drive the final ring-opening step to completion by using an excess of methyl lithium, and to use the mixture of isomeric hydrates obtained directly for kinetic studies without separation. As accompanying base-catalyzed aromatization/dehydration was unavoidable in the presence of excess methyl lithium, the starting material for kinetic studies in these cases did also contain some arene. Nevertheless, it proved straightforward to distinguish the reactions of the separate isomers using a combination of UV-visible spectrophotometry and ^{1}H NMR spectroscopy.

Attempts to prepare 3-substituted hydrates **8** with methoxy, thiomethoxy and dimethylamino substituents were unsuccessful. Monoepoxidation of commercially available unsubstituted 1,4-cyclohexadiene with *m*-CPBA, followed by allylic bromination in the presence of N-bromosuccinimide, yielded bromo-epoxide **9** (X = Br). Subsequent reaction of epoxide **9** (X = Br) with EtSH, MeOH or dimethylamine in acetonitrile solution in the presence of potassium carbonate gave epoxides **11** (X = EtS, MeO, Me_2N) in reasonable yields. However, all attempts to ring open purified epoxides **11** (X = EtS, MeO, Me_2N) yielded only parent arene, presumably a result of the rapid *in situ* aromatisation reaction of these highly activated hydrates.

Kinetic Analysis The acid-catalyzed dehydration reactions of arene hydrates **8a-d** and mixtures of alkyl hydrates **13a-d/14a-d** were followed in dilute $HClO_4$ solutions and acetic acid buffers by UV-Visible spectrophotometry at ionic strength, I = 0.5 ($NaClO_4$) at 25 °C. Figure 1 shows a representative UV spectrum for the reaction of hydrate **8a** (X = CO_2Me) in 6.38 mM $HClO_4$. The total substrate concentration that could be used was limited by the solubility of the arene dehydration product. As the reaction proceeded, a decrease in absorbance was observed at λ_{max} = 230 nm of hydrate **8a** together with a concomitant increase in absorbance at λ_{max} = 270 nm due to the formation of product arene **2** (X = CO_2Me). In the

case of hydrates **8a-d**, the slopes of semilogarithmic plots of (A_{obs}-A_{∞}) against time yielded values for k_{obs} (s^{-1}), the observed first order rate constants for dehydration. Non-linear least squares fitting of (A_{obs}-A_{∞}) data to a single exponential function yielded k_{obs} values in excellent agreement with those obtained from semilogarithmic linear first-order plots. The dehydration reaction of **8a** was also followed by ^{1}H NMR spectroscopy in 2.5 mM $DClO_4$ in D_2O at ionic strength, I = 0.5 ($NaClO_4$) at 25 °C (Supporting Information).

For the mixture of alkyl hydrates **13a-d/14a-d**, ^{1}H NMR spectroscopy of the dehydration reactions in deuterated phosphate buffers at pD 6.90 – 7.30 revealed the *ipso*-hydrates **14a-d** to be the more reactive in each isomeric pair (see Supporting Information). Although the presence of the *ipso*-isomer of *tert*-butylbenzene hydrate **14d** could clearly be inferred from ^{1}H NMR spectra in $CDCl_3$, only the 2-*tert*-butyl isomer **13d** was observed in the aqueous D_2O buffers due to the rapid reaction of **14d** under these conditions. As above, the dehydration reactions were also followed by UV-Visible spectrophotometry in dilute $HClO_4$ solutions and acetic acid buffers at ionic strength, I = 0.5 ($NaClO_4$) at 25 °C. At the lowest pH values, the dehydration of the more reactive *ipso*-hydrates **14a-c** was complete before acquisition of the first absorbance measurement and non-linear least squares fitting of (A_{obs}-A_{∞}) data, at the λ_{max} of hydrate, to a single exponential decay function yielded k_{obs} values for the 2-substituted hydrates **13a-c** only. For studies at higher pH values, the reactions of both isomeric hydrates could be followed and non-linear least squares fitting of (A_{obs}-A_{∞}) data, at the λ_{max} of hydrate, to a double exponential function yielded k_{obs} values for both the 2-substituted hydrates **13a-c** and the *ipso*-hydrates **14a** and **14c**. Although ^{1}H NMR spectra in D_2O buffers clearly revealed the presence of ~5% of ipso-isomer **14b**, reliable rate constants for dehydration could not be obtained in this case due to the small absorbance changes

observed. For the *tert*-butyl benzene hydrates, only the reaction of the 2-*tert*-butyl isomer **13d** could be followed by UV-visible spectrophotometry.

Kinetic measurements in acetic acid buffers showed no buffer catalysis, as was reported for benzene hydrate **1** (X = H) (see Supporting Information). [2] Small decreases in k_{obs} (s^{-1}) were generally observed upon increasing the total buffer concentration at a fixed buffer ratio presumably a result of a small medium effect. A short linear extrapolation of k_{obs} (s^{-1}) to zero-buffer concentration yielded the buffer-independent pseudo-first-order rate constant for dehydration at the relevant pH.

UV absorbance-time data, and kinetic fits to these data, are included in the Supporting Information. Tables S2, S5-6, S13, S22, S27, S37 and S45 report pseudo-first-order rate constants for dehydration of arene hydrates **8a-d**, **13a-d**, **14a** and **14c** at a range of pH values. Plots of first-order rate constants against acid concentration (Figures S5, S7, S23, S46, S56, S80 and S97) were linear with near-zero intercepts. The second-order rate constants for acid-catalyzed dehydration, k_H, were obtained from the slopes of these plots according to Eq 1.

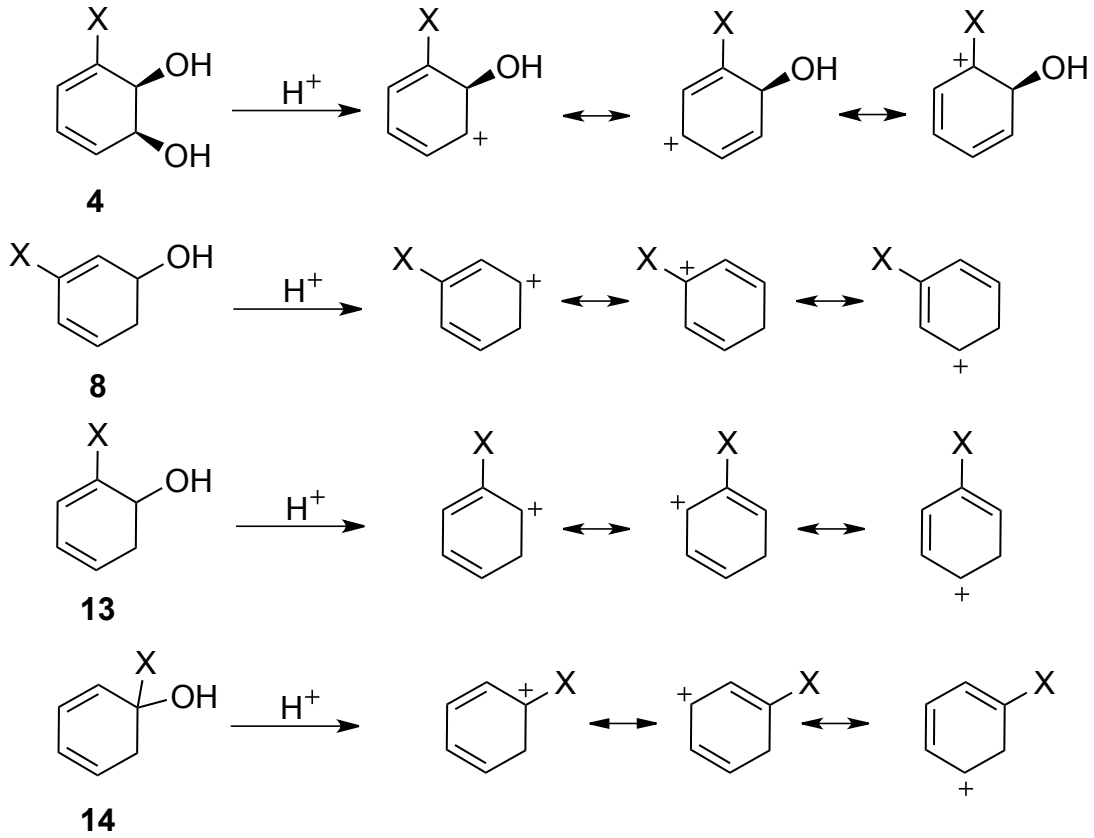
$$k_{obs} = k_H [H^+] \quad (1)$$

DISCUSSION

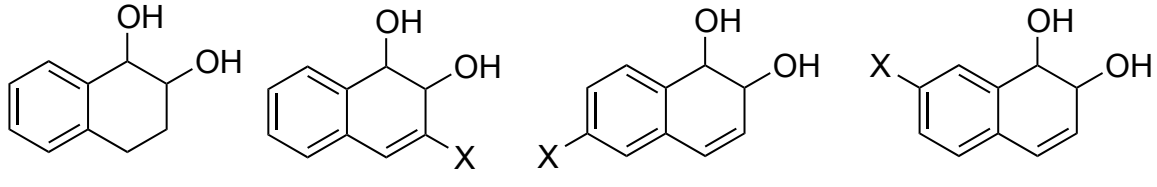
The absence of significant buffer catalysis and the linear dependence of k_{obs} values for dehydration on the concentration of hydronium ion are consistent with the *E*1 elimination mechanism in Scheme 1 involving a pre-equilibrium protonation on oxygen. In agreement with this mechanism, a ratio of k_{HClO4}/k_{DClO4} ~1,[9] was observed for the dehydration reaction of hydrate **8a**.

Table 1 lists the second-order rate constants obtained in this work for the acid-catalyzed dehydration of arene hydrates **8a-d**, **13a-d**, **14a** and **14c**. An increase in k_H is observed for hydrates bearing X-substituents with more negative Hammett σ-values as was also observed for the analogous dehydration reactions of 3-substituted-1,2-dihydrodiols **4**. This is consistent with dehydration *via* a carbocation intermediate as illustrated in Scheme 1. Rate constants for dehydration of the *ipso*-substituted hydrates **14a** and **14c** are 19- and 25-fold greater than for the 2-substituted analogues **13a** and **13c**.

Scheme 4



This is likely due to the greater stabilization of the carbocation intermediate formed in the dehydrations of hydrates **14** by hyperconjugation from the alkyl X-substituent and also by +I inductive effects. As shown in Scheme 4, the carbocations directly formed upon loss of water from *ipso*-substituted hydrates **14** have the positive charge on the *ipso*-carbon thus hyperconjugative interactions with a C-H of the methyl or ^{i}Pr-substituents is possible. By contrast, resonance structures for the carbocations formed from 2-substituted hydrates **13** place the positive charge at least one atom away from the carbon bearing the X-substituent. The +I inductive effect of an alkyl substituent will also be larger when directly attached to the carbocation centre, and could contribute ~ 5-10 fold of the increase in reactivity for the *ipso*-hydrates **14** based on a comparison of σ_m and σ_p values for methyl and ^{i}propyl-substituents. Notably, the degree of stabilization afforded by a 'non-aromatic' hyperconjugative interaction with an internal or external σ-C-H bond, is substantially smaller than by hyperconjugative aromatic stabilization within the ring of the benzenium intermediate (*cf.* k_{cis}/k_{trans} = 4500 for benzene-1,2-dihydrodiols[5b, 5f]). The equivalent 'non-aromatic hyperconjugative interaction' within the ring is reflected by the significantly lower k_{cis}/k_{trans} ratio of 5.7[5f] for acid-catalyzed dehydration of the dihydrodiol **15** of 3,4-dihydronaphthalene *via* a benzylic carbocation.



15 **16** **17** **18**

As mentioned earlier, Boyd and More O'Ferrall reported a large negative Hammett ρ-value of –8.2 for the acid-catalyzed dehydration of a large series of 3-substituted-1,2-*cis*-dihydrodiols **4**.[4] This value lies in the typical range of ρ = –2.5 to –12 for electrophilic aromatic substitution, which involves the formation of similar benzenium carbocation

intermediates. Notably, the best straight line fit through this data was based on a correlation with σ_p rather than σ^+. The correlation of log k_H^X values for thirteen 3-X-1,2-*cis*-dihydrodiols **4** versus σ_p in the range –0.24 to +0.46 gave $\rho = -8.2$ ($R^2 = 0.98$), however correlation with σ^+ for the same X-substituents gave $\rho = -4.7$ ($R^2 = 0.90$). This was interpreted as resulting from an imbalance in the development of resonance and inductive effects at the carbocation-like transition state. The cationic charge is initially developed remote from the substituent (Scheme 4) and requires that five carbon atoms lie in a plane for direct mesomeric interaction with the X-substituent. Lack of planarity would hinder the expression of resonance effects, however this would not be expected to have an impact on inductive effects. More recently, a similar analysis of the acid-catalyzed dehydration reactions of naphthalene-1,2-dihydrodiols revealed that the 3-substituted diols **16**, for reaction at the 1-hydroxyl group, show similar behavior to the 3-X-benzene-1,2-dihydrodiols **4** with surprisingly weak resonance interactions of +M substituents (MeO, Me).[5d] However, the acid-catalyzed dehydration reactions of 6- and 7-substituted naphthalene-1,2-dihydrodiols **17** and **18** are well-correlated by σ^+ for reaction of the 1- and 2-hydroxyl groups, respectively. An alternative interpretation of the reduction in resonance for **4** and **16** was suggested based on the steric 'interference' of the adjacent hydroxyl group on conjugation of +M substituents with the carbocation centre. Importantly, 'hyperaromatic' interactions can be ruled out as the basis of the apparently poor direct resonance interactions for the reactions of diols **4** and **16**, due to the observation of unimpaired resonance in **17** and **18**.

By contrast with 3-X-benzene-1,2-dihydrodiols **4**, the best linear fit through the data for the arene hydrates results from a correlation with σ^+ rather than with σ_p (Figure 2). The correlation of log k_H^X values (●) for hydrates **1a**, **8a-d, 14a and 14c** versus σ^+ in the range –0.31 to +0.48 gave $\rho = -6.52$ ($R^2 = 0.994$), however correlation with σ_p for the same X-substituents gave $\rho = -8.13$ ($R^2 = 0.929$) (Figure S98). Although hydrates with strong +M

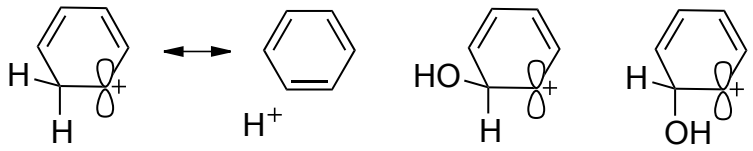
substituents (*e.g.* X = MeO) could not be isolated and studied, the Hammett correlation in Figure 2 covers a similar range of σ-values as used in the analogous correlation for *cis*-1,2-dihydrodiols **4**. The observation that data for the *ipso*-hydrates **14**, which require no rearrangement to enable direct interaction of substituent and carbocation centre, fits in the same correlation with data for the 3-X-hydrates **8a-d** suggests no imbalance in the development of resonance/inductive effects in this case. The 3-X-hydrates **8a-d** require that three atoms remain in the same plane for direct resonance interaction with the substituent, and the better correlation with σ^+ rather than with σ_p suggests that this planarity is achieved at the transition state.[10] Unsurprisingly the data for the 2-substituted hydrates **13a-d** (■) show significant negative deviations from this correlation line with σ^+. Resonance structures for the carbocation formed from these hydrates (Scheme 4) do not permit the direct interaction with the X-substituent.

Notably, the data points for hydrates **8a** and **8d** with +M and –M substituents, respectively, show positive and negative deviations in the poorer correlation of log k_H^X values versus σ_p (Figure S98). For the hydrates, the presence of a 3-phenyl substituent increases k_H by 11.6-fold whereas for the analogous diols a decrease in k_H by 1.7–fold is observed. This is presumably a reflection of the direct resonance interaction present for the hydrates, which cannot occur in **4** either due to difficulties in achieving planarity, and an associated imbalance in resonance/inductive effects at the transition state, or steric hindrance of the direct mesomeric interaction.

Acid-catalyzed dehydration of the arene hydrates is $3.7 \times 10^2 - 6.9 \times 10^4$ -fold faster than of analogous 3-X-*cis*-1,2-dihydrodiols **4** (Table 2). This is partly a manifestation of the destabilizing effect of the inductively electron withdrawing β~hydroxy substituent on the carbocation formed in the diol dehydration reaction. Assuming this destabilization is reflected by the 3.5×10^3-fold decrease in k_H observed for unsubstituted benzene-1,2-*cis*-

dihydrodiol **4** (X = H) relative to benzene hydrate **1** (X = H), the remaining variation in the k_{hyd}/k_{diol} ratios largely reflects the variation in direct mesomeric and inductive effects of the X-substituent for the hydrates versus diols. It is likely that the stabilization afforded by an aromatic hyperconjugative interaction from a β-hydrogen within the ring is possible for all hydrates due to the presence of the β-CH_2 group in all cases (*cf.* **19**, Scheme 5) .[11] In the corresponding diols, 'hyperaromatic stabilization' could only be realized in carbocation **20** formed from the *cis*-dihydrodiols and not the *trans*-analogues **21** as the latter has a β-OH rather than a β-hydrogen in a position to overlap with the vacant orbital at the carbocation centre.

Scheme 5



19 **20** **21**

In summary, second order rate constants for acid-catalysed dehydration have been measured for a range of monocyclic arene hydrates. A Hammett correlation of the data for 3-substituted hydrates **8** and *ipso*-hydrates **14** shows a better correlation with σ^+ rather than with σ_p, which is the opposite to that observed for analogous 3-substituted *cis*-1,2-dihydrodiols **4**. This suggests that planarity is achieved between the dienyl carbons and carbocation centre in the benzenium intermediate formed from the hydrates, which permits resonance and inductive effects to act in concert.

EXPERIMENTAL

Materials and Preparation of Solutions

Deuterium oxide (99.9 % D) was purchased from Apollo Scientific Ltd. Deuterated perchloric acid (60 %, 99.5 % D) and deuterated chloroform (99.8 % D) were purchased from Aldrich Chemical company. Acetonitrile-d_3 (99 % D), acetone-d_6 (99 % D) and sodium deuteroxide (40 wt % 98+ %D) were purchased from Cambridge Isotope Labs. All commercially available reagents were used as received. Solvents were dried using an appropriate drying agent when required: diethyl ether, THF and DCM were dried on the solvent purification system. Dry ethanol was prepared by refluxing over magnesium turnings and iodine followed by distillation. Water and H_2O refer to high purity water with conductivity of 18 mΩ obtained from the 'Millipore' purification system.

Thin-layer chromatography was carried out on neutral aluminium oxide plates (Merck Art 5550) or silica plates (Merck 5554), visualised under UV irradiation (254 nm), potassium permanganate, or iodine staining. Preparative column chromatography was carried out using neutral aluminium oxide (Acros 50-200 micron) or silica gel (Fluorochem, 40-63 micron).

NMR samples were prepared in deuterated chloroform, deuterium oxide and acetone-d_6. Tetramethylsilane (TMS) was used as an internal reference in deuterated chloroform. ^1H and ^{13}C NMR chemical shifts in $CDCl_3$ are reported relative to $CHCl_3$ at 7.27 ppm and 77.0 ppm respectively. In D_2O, ^1H NMR chemical shifts are reported relative to HOD at 4.67 ppm. In acetone-d_6, ^1H NMR chemical shifts are reported relative to acetone-d_5 at 2.17 ppm respectively. All chemical shifts are given in ppm and coupling constants in Hz. Splitting patterns are described as singlet (s), doublet (d), double-doublet (dd), triplet (t), quartet (q), double multiplet (dm), pseudo-quartet (pq) or multiplet (m).

Stock solutions of perchloric acid were prepared by dilution and titration of the commercially available concentrated solutions. Stock solutions of sodium phosphate (Na_2HPO_4 and

NaH_2PO_4) were prepared by dissolving dry commercial samples of Na_2HPO_4 and NaH_2PO_4 in distilled, deionised Millipore water to a final concentration of 1 M. Phosphate buffers were then prepared by mixing the stock solutions of Na_2HPO_4 and NaH_2PO_4 in water with addition of $NaClO_4$ to give solutions of buffer at various acid/base ratios and I = 0.5 ($NaClO_4$). Stock solutions of sodium acetate were prepared by dissolving the dry commercial sample in distilled, deionised Millipore water to a final concentration of 1 M. Stock solutions of acetic acid were prepared by dilution and titration of the commercial concentrated solutions. Acetic acid buffers with 90 and 75 % free base (FB) and I = 0.5 ($NaClO_4$) were prepared by mixing the stock solutions of sodium acetate and perchloric acid in water with the addition of $NaClO_4$. Further acetic acid buffers were prepared by mixing the stock solutions of sodium acetate and acetic acid in water with the addition of $NaClO_4$ to give 0.5 M solutions with 50, 25 and 10 % FB and I = 0.5 ($NaClO_4$). The buffer dilutions used were 0.2, 0.1, 0.05 and 0.025 M.

Stock solutions of deuterated perchloric acid were prepared by dilution and titration of the commercial concentrated solutions. Stock solutions of buffers, Na_2DPO_4 and NaD_2PO_4, were obtained from sodium phosphate monobasic and dibasic by exchanging the hydrogen atoms for deuterium. This was achieved by dissolving the salts in D_2O, followed by removal of solvent under reduced pressure. The process was repeated five times and the salts were freeze dried. Phosphate buffers were prepared by mixing stock solutions of Na_2DPO_4 and NaD_2PO_4 in D_2O with addition of $NaClO_4$ to give solutions of buffer at various acid/base ratios and I = 0.5 ($NaClO_4$).

For the UV spectrophotometric kinetics, the stock substrate solutions were prepared in acetonitrile to a final concentration of 50 mM. The internal standards for the ^1H NMR analysis were prepared by accurately weighing either methanol or sodium 3-(trimethylsilyl)propanoate into a glass vial and accurately transferring sodium perchlorate

solution (500 μL, 0.5 M in D_2O) to this by microlitre syringe to give a final concentration of 1 M.

pH Measurement

The pH of buffered solutions was determined at 25 ºC using a MeterLab™ PHM 290 pH-Stat Controller equipped with a radiometer (pH 4 - 7 - 10 @ 25 ºC) combination electrode (type pHC4006) with a saturated calomel solution as reference. The electrode filling solution used was a saturated solution of lithium trichloroacetate (LiTCA) in place of regular saturated KCl solution.

LiTCA was prepared by dissolving trichloroacetic acid (52.5 g, 0.32 mol) in ice-cold water. [12] Lithium hydroxide monohydrate (12.4 g, 0.30 mol) was added slowly. The solution was allowed to stir for 10 minutes to ensure the salts had dissolved. The excess trichloroacetic acid was extracted with diethyl ether (5 × 50 mL). The aqueous solution was filtered through sintered glass and the water was removed *in vacuo*. The salts were dried under vacuum over four days. Elemental analysis was conducted to determine purity: expected C: 14.19, H: 0.00, Cl: 62.82. Found C 14.08, H: 0.00, Cl: 61.13.

A saturated solution (~5 M) was prepared using Millipore water. The saturated KCl was removed from the probe and all traces of KCl were removed by careful washing using Millipore water. The saturated LiTCA solution was added and the probe was allowed to stand for 1 hour. This solution was replaced 5 times, allowing the probe to stand for 5 minutes between each washing. After the final solution of LiTCA was added the probe was allowed to stand in pH 1 buffer overnight and then calibrated as described below.

The pH meter was calibrated with standard buffer solutions of pH 4.00 (phthalate buffer), 7.0 (phosphate buffer) and 10 (borate buffer). For calibration at lower pHs, a 0.1 M solution of HCl in KCl was freshly prepared and used directly.

$$[H_3O^+] = 10^{-pH(obs)} / \gamma_H \qquad \textbf{Eq 2}$$

The hydronium ion concentration at any pH was calculated using Eq 2, where $\gamma_H = 1.02$ is the apparent activity coefficient of hydronium ion under our experimental conditions. To determine a value for γ_H, a series of dilutions of standardised 1M $HClO_4$ were made to give a titrimetrically known series of $HClO_4$ solutions in the concentration range 0.001 – 0.1 M at ionic strength, I = 0.5 ($NaClO_4$). A plot of $10^{-pH(obs)}$ against the concentration of hydronium ion yield the activity coefficient as slope.

^{1}H NMR Analysis of the Dehydration of Arene Hydrates

A typical experiment involved accurately transferring buffer or dilute acid solution (890 μL) by microlitre syringe to a reaction vial. To this solution internal standard stock solution (10 μL) was added. Substrate stock solution (100 μL) was added to the buffered solution and mixed vigorously. An aliquot of the reaction mixture (750 μL) was transferred to an NMR tube and the reaction was followed *in situ* by ^{1}H NMR spectroscopy. ^{1}H NMR spectra of the solution dehydration reactions of hydrates were recorded on an Oxford Varian Inova 400 or 500 spectrometer with a relaxation delay of 18 s, sweep width of 7996.8 Hz, and acquisition time of 6 s, and a 90º pulse angle. Spectra were run with 32 transients with a total acquisition time of 12 minutes 32 seconds.

UV-Visible Spectrophotometry

For UV-visible spectrophotometric analyses, Cary 100 and Cary 50 spectrophotometers were used. Quartz cuvettes (1 cm, 3 mL) fitted with teflon caps were employed and the temperature in the cell compartment was maintained at 25.0 ± 0.1 ^oC.

A typical kinetics run was carried out by accurately pipetting 3 mL of buffer or $HClO_4$ solution into a cuvette, and allowing it to equilibrate at 25 °C. The reaction was initiated by addition of the substrate stock solution (30 μL) in acetonitrile by microlitre syringe. Mixing

was achieved by inverting the cuvette three times. The cuvette was then inserted into the spectrophotometer; and the collection of the absorbance versus time data was started at this point. The kinetic reactions were generally followed at the λ_{max} of the reactants and where possible the kinetic reactions were followed for both the appearance of products and the disappearance of the reactants.

Data Analysis

The results obtained were analysed using the Sigma-plot (Version 8.02) statistics software. The absorbance versus time data were fitted to a single exponential equation (Eq 3) and/or the double exponential equation (Eq 4). .

$$y = y_0 + Ae^{-bx} \qquad \textbf{Eq 3}$$

$$y = y_0 + Ae^{-bx} + Ce^{-dx} \qquad \textbf{Eq 4}$$

The first order rate constants are obtained as the parameters *b* and *d* in the equations above.

For data that fitted well to Eq 3, the first order rate constant was also obtained from the slope of a semi-logarithmic plot of the change in absorbance against time (ln (A_t-A_∞) against time). Reactions were typically followed for approximately 10 half-lives so as to reliably estimate A_∞.

For reactions performed in acetic acid buffers, the observed pseudo-first-order rate constants at a given p*H* are quoted both as the mean of the values at different buffer concentrations and also as the intercept, k_{int}, of the plot of k_{obs} against buffer concentration at a fixed p*H*.

Supporting information

The details of synthetic procedures and the kinetic data are included in the Supporting Information.

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[8] The mono-epoxides used in the final ring-opening step were a mixture of diastereomers.

[9] A rate constant of $k_{DClO4} = 2.0\ (\pm 0.2) \times 10^{-4}\ s^{-1}$ was determined by ^1H NMR spectroscopy for the reaction of **8a** in 2.5 mM $DClO_4$ in D_2O solution. A rate constant of $k_{HClO4} = 2.25\ (\pm 0.08) \times 10^{-4}\ s^{-1}$ can be calculated for reaction of **8a** in 2.5 mM $HClO_4$ in H_2O solution from the second order rate constant for acid-catalyzed dehydration $k_H = 0.0899\ M^{-1}s^{-1}$, thus giving a $k_{HClO4}/\ k_{DClO4}$ of ~1.

[10] Preliminary calculations using Gaussian 2003 B3Lyp6-31g** level of theory have shown that the presence of the hydroxyl group in the carbocation formed from diols **4** results in puckering of the two saturated carbons relative to the diene, whereas the hydrate carbocation centre is co-planar with the diene.

[11] Our preliminary calculations using Gaussian 2003 B3Lyp6-31g** level of theory have shown that the most stable conformation for all of the hydrates places the hydroxyl group in an axial position, thus permitting an 'aromatic' hyperconjugative interaction between the developing vacant carbocation p-orbital and one of the β-CH_2 hydrogens in the ring.

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